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Enterovirus D68 outbreak detection through a syndromic disease epidemiology network

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ABSTRACT

Background: In 2014, enterovirus D68 (EV-D68) was responsible for an outbreak of severe respiratory illness in children, with 1,153 EV-D68 cases reported across 49 states. Despite this, there is no commercial assay for its detection in routine clinical care. BioFire® Syndromic Trends (Trend) is an epidemiological network that collects, in near real-time, deidentified. BioFire test results worldwide, including data from the BioFire® Respiratory Panel (RP).

Objectives: Using the RP version 1.7 (which was not explicitly designed to differentiate EV-D68 from other picornaviruses), we formulate a model, Pathogen Extended Resolution (PER), to distinguish EV-D68 from other human rhinoviruses/enteroviruses (RV/EV) tested for in the panel. Using PER in conjunction with Trend, we survey for historical evidence of EVD68 positivity and demonstrate a method for prospective real-time outbreak monitoring within the network.

Study design: PER incorporates real-time polymerase chain reaction metrics from the RPRV/EV assays. Six

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institutions in the United States and Europe contributed to the model creation, providing data from 1,619 samples spanning two years, confirmed by EV-D68 gold-standard molecular methods. We estimate outbreak periods by applying PER to over 600,000 historical Trend RP tests since 2014. Additionally, we used PER as a prospective monitoring tool during the 2018 outbreak.

Results: The final PER algorithm demonstrated an overall sensitivity and specificity of 87.1% and 86.1%, respectively, among the gold-standard dataset. During the 2018 outbreak monitoring period, PER alerted the research network of EV-D68 emergence in July. One of the first sites to experience a significant increase, Nationwide Children's Hospital, confirmed the outbreak and implemented EV-D68 testing at the institution in response. Applying PER to the historical Trend dataset to determine rates among RP tests, we find three potential outbreaks with predicted regional EV-D68 rates as high as 37% in 2014, 16% in 2016, and 29% in 2018.

Conclusions: Using PER within the Trend network was shown to both accurately predict outbreaks of EV-D68 and to provide timely notifications of its circulation to participating clinical laboratories

1. Background

Enterovirus-D68 (EV-D68) is a member of the family *Picornaviridae* belonging to the species *Enterovirus D,* having characteristics of both the *Enterovirus* (EV) and *Rhinovirus* (RV) species within the family [1]. EV-D68 respiratory illness in children typically presents with mild-to-severe clinical symptoms, such as runny nose, fever, cough, wheezing, and dyspnea, which may lead to hospitalization requiring intensive care [2,3]. Children with an underlying medical condition, such as asthma or recurrent wheezing, are at particular risk of severe EV-D68 infection [4]. In recent years, there is accumulating evidence to support that EV-D68 is associated with acute flaccid myelitis (AFM) in children [5–9]. In 2014, clinical laboratories verified 1153 EV-D68 cases across 49 states [10,11], and significant outbreaks were also reported in Europe and Asia [12–15]. Isolated clusters of EV-D68 have continued to be identified since 2014, with a majority of them detected in 2016, corresponding with an upsurge in AFM cases [16,17].

Despite EV-D68's emergence as a major cause of severe respiratory disease worldwide, there is no commercial assay for its detection in routine clinical care. In 2015, the US Food and Drug Administration gave Emergency Use Authorization to the Centers for Disease Control and Prevention's (CDC) real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assay for clinical diagnosis of EV-D68 [18]. A number of clinical laboratories capable of performing high complexity tests have also developed molecular assays to detect EV-D68 within their institutions [19–22], although these tests are not typically employed unless EV-D68 is suspected through local reporting of the virus or diagnosis of AFM.

EV-D68 is among the diverse *Enterovirus* genus pathogens containing more than 150 RV and 100 EV serotypes [23] detected by multiplex platforms which target most known etiological agents of respiratory disease [24–29]. Among these is the BioFire® FilmArray® Respiratory Panel version 1.7 (RP), a multiplex nested RT-PCR test capable of detecting up to 20 respiratory pathogens [24]. There are six nested RT-PCR assays in the BioFire RP which make up the pan RV/EV assay. These assays target the 5' untranslated region (UTR) of the *Picornavirus* genome. Due to near homogeneity of the 5' UTR, BioFire RP cannot differentiate RV and EV and reports a generalized RV/EV pathogen determination.

In 2016, BioFire launched a research epidemiology software service, BioFire® Syndromic Trends (Trend) that automatically collects de-identified test results from BioFire Systems at participating research clinical sites [30]. This network spans the US and is also dispersed worldwide. The connected systems have sent over 600,000 RP patient test results to the database, with the earliest results dating back to 2012.

2. Objectives

We design a model, Pathogen Extended Resolution (PER) to identify EV-D68 among RP data obtained from routine testing of patients with respiratory infections, to predict outbreaks of EV-D68. We use data from 1619 samples, which have been independently evaluated by EV-D68 gold-standard laboratory methods, to build and validate the model. We then use data from the Trend database to identify potential cases of EV-D68 among the US respiratory disease patient population testing positive for RV/EV. Using Trend in this capacity could provide a potential early-warning system of EV-D68 predictions from BioFire RP tests as well as augment public health response through increased situational awareness of novel pathogens [31–33].

3. Study design

3.1. Data

There are two independent data sources used in this study: A dataset containing BioFire RP data from clinical samples previously tested for EV-D68 by gold-standard molecular methods was used to train the PER model, and the Trend dataset consisting of de-identified clinical BioFire RP tests used for regional predictions.

The training data samples were collected and tested on BioFire RP as part of routine patient care and were positive for RV/EV. These samples were evaluated for EV-D68 independently by each clinical laboratory for investigation purposes amid known periods of significant EV-D68 circulation. In some cases, the samples were selected based on time and location of increased severity or incidence of respiratory disease or AFM. Samples were identified at the participating sites using methods approved by their respective Institution Review Boards. Four sites provided the clinical adjudications for each sample to BioFire for

Table 1
Contributing sites used in algorithm development and comparator method summary. †Sites in the Train/Test dataset are used in model development. * Sites in the validation set ware not used in the training process.

Site	Location	N	Collection Period	Comparator Assay (Citation)
† Albany Medical Center	Albany, NY, USA	45	Aug.-Sept. 2014	Wadsworth Center RT-PCR Confirmed, Sequencing [[34]]
† Children's Hospital Colorado	Denver, CO, USA	891	Jul. 2014-Sept. 2016	2014 Outbreak – CDC RT-PCR EV assay followed by sequencing [35], After 2014 Outbreak - Wylie RT-PCR Assay [19]
† Children's Hospital of Los Angeles	Los Angeles, CA, USA	70	Sept. 2016	Wylie RT-PCR Assay [19]
† Children's Mercy Hospital	Kansas City, MO, USA	532	Jul.-Dec. 2014	CDC real time RT-PCR assay [3], Sequencing
* Primary Children's Hospital	Salt Lake City, UT, USA	22	Aug.-Sept. 2014	CDC confirmed
* University Medical Center	Groningen, NL	59	Jul. 2014-Sept. 2016	Lab Developed RT-PCR test [36,37]

model training and testing. The remaining two sites calculated performance results at their institutions for validation of the final model. A summary of each gold-standard comparator method used by contributing sites as well as sample sizes can be found in Table 1.

The Trend dataset, used here to perform regional EV-D68 predictions and real-time monitoring, consists of BioFire RP test results from participating clinical laboratories across the four US CDC regions (Table S1). The number of contributing states and sites within each region have increased each year. The de-identified test results are from a combination of pediatric and general inpatient and outpatient sites, as well as reference laboratories [30]. 138,614 RP test results that were positive for RV/EV from the period July 2014 through December 2018, were exported for evaluation by PER. There was not sufficient data reported prior to 2014 within the Trend system to accurately study EV-D68 positivity rates.

Nationwide Children's Hospital (NCH, Columbus Ohio) investigated the 2018 EV-D68 outbreak detected in real time by PER through evaluating 1017 samples positive for RV/EV with a real-time RT-PCR laboratory developed test (LDT) for EV-D68 identification [22]. These test files were de-identified and exported to BioFire for PER evaluation and LDT result comparison. Additionally, NCH performed discrepancy analysis on select samples using nucleotide sequencing of the VP1 region [22] and tested for the presence of Enterovirus using a RT-PCR assay [38].

3.2. PER model development

PER is a novel algorithm designed to classify patient tests as either EV-D68 positive or negative. Fluorescence measurements from the BioFire RP v1.7 RV/EV assays were used as inputs to our model. For each test, the BioFire® FilmArray® Software records three metrics from the second stage PCR [24]: 1) crossing threshold (Ct) of the amplification curve indicates the cycle in which the fluorescent signal rises above background, 2) melting temperature (Tm) is the temperature at which the DNA amplicon has the greatest dissociation rate; and 3) the maximum fluorescent signal (Fmax) acquired during PCR amplification. Only the Tm values are available to the clinical operator of the BioFire instrument. Each of these metrics is reported for three replicate amplicons in the BioFire test. We calculated the test replicate medians of the three metrics from four of the six RV/EV assays thus resulting in 12 model inputs. The two RV/EV assays excluded from the model target a sequence found in most *Enteroviruses* that is not present in EV-D68 and were primarily negative.

Because of its broad capability in separating data belonging to two groups, we trained a Support Vector Machine (SVM) classification model with a radial basis function kernel [39]. Four sites provided a total of 1538 BioFire RP tests adjudicated as positive or negative for EV-D68. These RP tests were used to train and test the SVM (Table 1). To estimate the model performance we employed a stratified 10-fold training and testing schema in which the data from the four sites were randomly distributed across each fold. Area under the receiver operating characteristic curve (AUC) was used to estimate performance on each fold. This metric is frequently used to evaluate binary classification models where a value of 1 indicates perfect sensitivity and specificity. The SVM was then trained on all 1538 gold-standard results to create the final model, PER, and was applied to the validation dataset. The model efficacy for each institution was measured using sensitivity, specificity and accuracy.

3.3. Real-time outbreak monitoring

Among the 616,108 total BioFire RP tests in the Trend dataset, we applied the PER algorithm to the 138,614 RV/EV positives for historical analysis and real time outbreak monitoring during 2018. We calculated weekly EV-D68 rates among all RP tests for each site. To reduce noise due to low sample sizes, we only applied PER to sites with more than 12 RP tests in a given week. Using these site rates, we determined regional means and 95 % confidence intervals (CI). To identify potential outbreaks, regional baselines were established as the normal background rate of predicted EV-D68 positivity. We calculated each regional historical baseline prior to 2018 by averaging the regional site EV-D68 predicted positivity, adding to that three standard deviations. Known outbreak periods of 2014 [2] and 2016 [37] were excluded. Predicted positivity rates greater than three standard deviations over baseline for at least three consecutive weeks were considered significant periods of predicted EV-D68 transmission. Due to regional variability, EV-D68 predicted positivity at the national level was not reported, nor was a national baseline created in this analysis.

4. Results

4.1. Model evaluation against the training set

Using the stratified 10-fold training and testing schema, the mean AUC across the 10 folds was 0.91 ± 0.02 standard deviations, with values ranging from 0.88 to 0.97. The model efficacy from the six clinical institutions that contributed data to the training, testing and validation datasets are reported in Table 2. In this dataset, 489 (30.2 %) samples were adjudicated as positive for EV-D68 by molecular methods. The PER model had 87.1 % sensitivity and 86.1 % specificity across all sites and ranged from 77.2 to 95.7% sensitivity and 84.0 to –94.4 % specificity. Among the 59 samples obtained from University Medical Center Groningen were 31 typed strains of RV/EV species including one echovirus, three EV, and 27 human rhinovirus (HRV) serotypes. PER predicted EV-D68 positivity for the EV-D68, EV-C104, EV-C109, and HRV-C13 type strains. PER returned a negative predicted value for EV-D68 (1/23 samples), echovirus 11, and the other 26 HRV serotypes in the sample set.

4.2. Real-time outbreak alert and confirmation

Population-level EV-D68 rates for the four major CDC regions

Table 2
PER performance evaluation.

Clinical Site	N	Sensitivity		Specificity		Accuracy	Primary Comparator
Albany Medical	45	16/18	88.90 %	24/27	88.90 %	88.90 %	Wadsworth Center RT-PCR Confirmed, Sequencing (34)
Colorado Children's Hospital	891	88/114	77.20 %	653/777	84.00 %	83.20 %	2014 Outbreak – CDC RT-PCR assay followed by sequencing [35], After 2014 Outbreak - Wylie RT-PCR Assay (19)
Children's Hospital Los Angeles	70	15/16	93.80 %	48/54	88.90 %	90.00 %	Wylie RT-PCR Assay [19]
Children's Mercy Hospital	532	280/306	91.50 %	205/226	90.70 %	91.20 %	CDC real time RT-PCR assay (3)
Primary Children's Hospital	22	10/12	83.30 %	9/10	90.00 %	86.40 %	CDC Confirmed
University Medical Center Groningen	59	22/23	95.70 %	34/36	94.40 %	94.90 %	Laboratory Developed Test, (36, 37)
	1619	**431/489**	**87.10 %**	**973/1130**	**86.10 %**	**86.70 %**	

within the Trend dataset were established for use in real-time monitoring. Baseline rates were found to be 2.8 % in the Midwest, 2.8 % in the Northeast, 3.3 % in the South, and 2.2 % in the West. In 2018, regional EV-D68 predicted mean positivity rose above all four baseline values, and 37 clinical Trend research sites were alerted of potential pathogen circulation. This increase in activity was first associated with NCH (Columbus, Ohio). The hospital was notified in July (epidemiological or "epi" week 29) of the possible outbreak at their site (Fig. 1), and in response began routine clinical testing for EV-D68 via a RT-PCR LDT [22]. This testing continued until the outbreak subsided.

Between June 13th and October 16th, 2018 (epi weeks 24–41), NCH tested up to 245 clinical samples per week (3299 total) with the BioFire RP as part of routine clinical care, finding 1521 (46.1 %) positive for RV/EV. Over the course of the outbreak, NCH tested 1017 of the RV/EV positive samples using their EV-D68 LDT [22], confirming 399 of these as positive. To determine outbreak onset, 135 samples were tested with the LDT from the five weeks prior to the alert. Per evaluation of the 1017 samples returned 370 positive EV-D68 predictions. No positives were predicted by PER nor detected by the NCH LDT in the week of June 13 (epi week 24). Following this, a steady increase continued, peaking at 64 NCH LDT detections and 60 PER predictions per week at the end of August.

PER and NCH test concordance was overall 91 % in agreement (85 % sensitivity, 95 % specificity). NCH evaluated the 31 PER false positive samples which were retrieved for further analysis with an in-house RT-PCR test specific for *Enterovirus* [38]. Sixteen of these samples were confirmed positive for *Enterovirus*, with an average Ct value of 35.4 (range 31.6–38.3). Among the 60 false negative PER samples, the in-house EV-D68 LDT had an average Ct value of 30.2 (range 16.5–39.8), and 27 samples sequenced for the VP1 gene were identified as EV-D68. In evaluation of the BioFire RP Ct values, we found the 60 PER false negative tests to have an average RV/EV Ct value 2.7 cycles earlier than the concordant positive tests. Of the 31 PER false positives, the average Ct value was 3.7 cycles later than the concordant true positive group.

4.3. PER test application to historical trend data

In our evaluation of historical Trend data, he regional 3-week moving average predicted positivity rates of EV-D68 exceeded their respective baselines in all regions in 2014, 2016 (with the exception of the Midwest) and again in 2018 as shown in Fig. 2. The Midwest experienced the highest mean and site maximum EV-D68 predicted positivity in 2014 and 2018 (Table 3), but also presented the lowest positivity of all regions in 2016. Average outbreak durations as well as regional mean and maximum predicted EV-D68 rates were generally higher during the 2014 outbreak. The total predicted EV-D68 detections for the three outbreak periods were 1485 in 2014 with 11 states reporting, 1556 in 2016 with 20 states reporting and 2602 in 2018 with 22 states reporting.

5. Conclusions

Nowhere in the world is EV-D68 infection required to be reported to public health, and no widespread real-time epidemiology systems exist to detect potential outbreaks or to understand the burden of disease. The responsibility of both testing and voluntary reporting lies on individual laboratories. Furthermore, testing is generally limited to periods of high suspicion of disease due to local reports of pathogen circulation or AFM. The PER model described in this paper uses data obtained from routine testing of patients with respiratory infections, and in combination with the Trend network, was shown to accurately predict outbreaks of EV-D68. The general PER method of subtyping using PCR fluorescence measurements may also be extended to other pathogens, for example novel influenza A subtypes may be predicted among the five RP influenza A assays, and could support existing monitoring systems for pathogen outbreak detection.

Using 1619 samples externally validated for EV-D68 positivity, PER had a sensitivity of 87 % and a specificity of 86 %. We observed significant variability among sites with accuracy ranging from 83.2 to 94.9%. This may be attributed to an uneven distribution of gold-standard data used in development of this model including geographic location, time, sample size, and comparator method. No discrepant analysis could be conducted as most samples from the training dataset were no longer available. When applied to the Trend dataset, PER estimated regional EV-D68 positivity averages as high as 37 % among patients presenting respiratory symptoms selected for RP testing, with significant rates of detection in 2014, 2016, and 2018. Differences in regional variation may indicate environmental factors related to EV-D68 transmission dynamics as noted with other *Enteroviruses* [40]. When PER was used prospectively to alert clinical research collaborators of potential EV-D68 circulation, it was the primary notification system and allowed an individual site begin routine testing for EV-D68.

The BioFire RP was not specifically designed to detect EV-D68 and we explore the nuances of PER's inputs. The RP RV/EV assays used in PER targets the conserved 5' UTR region of the genome; however, there is sufficient genomic diversity within this region to allow for placement of RT-PCR assays targeting EV-D68 specifically [22]. *Enteroviruses* continually evolve and multiple clades have been found in circulation during single outbreak periods; although, recombination and clade identification is isolated to the capsid region outside of the 5' UTR

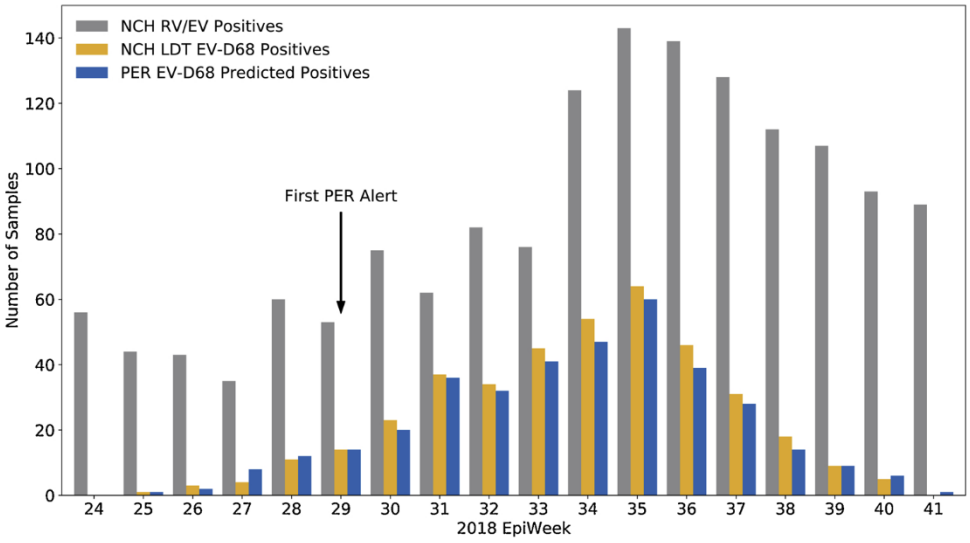

Fig. 1. EV-D68 detection at Nationwide Children's Hospital. RV/EV positive BioFire RP weekly test numbers from June (beginning epi week 24) through October 2018 (ending epi week 41) displayed with bars. BioFire RV/EV positive tests (grey), NCH laboratory developed EV-D68 positive tests (yellow), and PER predicted EV-D68 positive tests (blue) are shown. The date NCH first received an alert of potential EV-D68 outbreak was July 17, 2018 (epi week 29). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

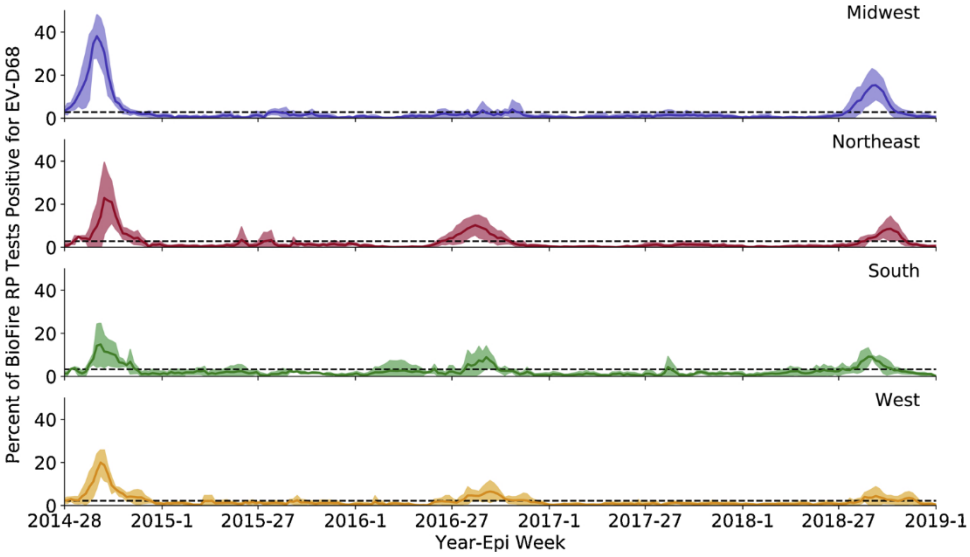

Fig. 2. Population level EV-D68 predicted positivity rates. Fig. 2: Graph displays PER predicted EV-D68 positivity (3-week moving average indicated by line and 95 % CI indicated by area) across the four US CDC regions as a percentage of all BioFire RP tests plotted by epi week. The dashed line denotes the regionally-specific EV-D68 predicted baseline.

Table 3
Regional mean and maximum site prediction values shown with predicted outbreak duration.

Year	Midwest	Northeast	South	West	Average Predicted Outbreak Duration
			(Mean, Max)		
2014	37 %, 47 %	23 %, 33 %	15 %, 25 %	20 %, 27 %	18 Weeks
2016	4 %, 14 %	10 %, 18 %	9 %, 17 %	6 %, 16 %	14.7 Weeks
2018	15 %, 29 %	8 %, 20 %	9 %, 12 %	4 %, 12 %	12.5 Weeks

[10,16,41–45]. The location of the RP RV/EV assay within the 5' UTR may contribute to the stability of the algorithm over time. Additionally, PER may be re-tuned as more samples become available. In evaluation of the NCH EV-D68 LDT and PER, the algorithm sensitivity appeared to be limited (85 %). The PER false negative tests exhibited robust EV-D68 LDT and BioFire RP Ct values, perhaps indicating moderate levels of pathogen within the sample. One cause for this disparity may be the presence of an additional RV/EV pathogen within the specimen which would likely interfere with the PER algorithm. Co-circulation of EV-D68 and other RV/EVs as seen during the 2018 NCH outbreak may support this hypothesis; however, isolates were not investigated. Due to these limitations, PER is not used for confirmation of EV-D68 in individual clinical samples; however, the algorithm demonstrates a novel mechanism for near real-time outbreak detection of a clinically important pathogen.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data access

Data used in this study has been aggregated to further protect both the privacy of the patient for publication and the identity of the contributing institutions. Access to data used in the study may be made available upon request.

CRediT authorship contribution statement

Lindsay Meyers: Conceptualization, Investigation, Methodology, Writing - original draft, Supervision, Resources. **Jennifer Dien Bard:** Investigation, Resources, Writing - review & editing. **Ben Galvin:** Methodology, Software, Validation, Formal analysis, Writing - original draft. **Jeff Nawrocki:** Methodology, Software, Validation, Formal analysis, Writing - original draft. **Hubert G.M. Niesters:** Investigation, Resources, Writing - review & editing. **Kathleen A. Stellrecht:** Investigation, Resources, Writing - review & editing. **Kirsten St. George:** Investigation, Resources, Writing - review & editing. **Judy A. Daly:** Investigation, Resources, Writing - review & editing. **Anne J. Blaschke:** Investigation, Resources, Writing - review & editing. **Christine Robinson:** Investigation, Resources, Writing - review & editing. **Huanyu Wang:** Investigation, Resources, Writing - review & editing. **Camille V. Cook:** Data curation. **Ferdaus Hassan:** Investigation, Resources, Writing - review & editing. **Sam R. Dominguez:** Investigation, Resources, Writing - review & editing. **Kristin Pretty:** Investigation, Resources, Writing - review & editing. **Samia Naccache:** Investigation, Resources, Writing - review & editing. **Katherine E. Olin:** Methodology, Software, Validation, Formal analysis, Writing - original draft. **Benjamin M. Althouse:** Writing - review & editing. **Jay D. Jones:** Conceptualization, Writing - review & editing. **Christine C. Ginocchio:** Writing - review & editing. **Mark A. Poritz:** Conceptualization, Writing - review & editing. **Amy Leber:** Investigation, Project administration, Conceptualization, Writing - original draft. **Rangaraj Selvarangan:** Investigation, Project administration, Conceptualization, Writing - original draft.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104262.

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